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SCANNING ELECTRON MICROSCOPIC STUDY OF THE FETAL BONES FOR DETERMINING THE AGE

ABSTRACT: To apply the scanning electron microscopic study in the forensic and historical anthropological study of the bones is very advantageous because no circumstantial preparation of the bone samples, bone sections or decalcified segments is needed.
Bone samples from the bone collection of our institute, sawn-out of the middle part of the femur of 25 human foetuses of different age (V-X lunar months old) were studied.
Tests were carried out by the electron microscope type TESLA BS 300 at 25 kV accelerating voltage on the fracture surfaces of the bones which had been covered by a 300 Å golden layer. In case of an unknown fetal skeleton it is impossible to determine the age with lunar month difference accuracy on the basis of the bone structure alone. However, statements about the qualification of crime from the forensic aspect can be made by approximate accuracy on the basis of the scanning electron microscopic study of the bones as well (whether the foetus was mature or immature; whether abortion or early birth happened).

KEY WORDS: Fetal bones — Scanning electron microscopic study — Determination of age — Application in forensic and historical anthropology.

INTRODUCTION

The historical study of the fetal bones on bone sections and decalcified histological segments is an applied method in medical expert practice and historical anthropological research (Ahlqvist and Damsten 1969, Amprino 1965a, Amprino and Bairati 1936, Amprino and Marotti 1964, Jowsey 1960, 1966, Kerley 1965, 1969).

The microscopic study of the bones for determining the age is carried out by considering the traits in qualitative and quantitative ways (Adams et al. 1970, 1971, Amprino 1955, 1965b, Arnold et al. 1966, Atkinson 1962, Barer and Jowsey 1967, Chalmers and Weaver 1966, Cook 1961, Demeter and Mátyás 1928, Dunnill et al. 1967, Enlow 1962, Frost 1966, Garn et al. 1967, Jowsey et al. 1965, Lacroix 1951, Schenk et al. 1969, Strandh 1960, Weel 1948). Demeter and Mátyás are of the opinion (1928) that studying the quantitative relations alone does not make it possible to differentiate human and animal bones and to determine the individual age. Qualitative differences have at least the same significance.

The knowledge about the osseous structure has great importance first of all from the forensic medical aspect because the histological picture of the fetal bones is totally different from that of the grown-ups (Balthazard and Müller 1921, Basset and Winell 1965, Bell 1956, Brash 1934, Cameron et al. 1964, Cameron 1967, Cohen and Harris 1968, Currey 1964, Demeter and Mátyás 1928, Dequeker et al. 1971, Felts 1959, Jowsey 1960, 1964, 1966, Kerley 1965, 1969).

A great number of authors studied the osseous structure of human foetuses and its development during the intrauterine life (Aho 1966, Hall 1971, 1972, Kember

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1960, Murray 1926, 1936, Owen and MacPherson 1963, Scherft 1972, Sissons 1956). Demeter and Mátyás (1928) made bone sections from the middle part of the femur of foetuses of V–X lunar months, which included the whole cross-section. They found that in the initial phase of the process of ossification as a first step fine bone plates were formed, surrounded by bone cells. Following that, blood-vessels grow into the osseous matrix from outside (except the dorsal area). The venous network consists of mostly orbicularis and longitudinal blood-vessels. But also blood vessels which are slanting, tangential and perpendicular to the surface may occur. Near linea aspera the venous network is not very regular. Here, mostly radially running vessels occur. In the beginning, the blood-vessels are remarkably wide and do not form osteons around themselves. The cross-section of the bone is not homogeneous yet. Inner and outer zones can be differentiated whose structure is different from each other. The human fetal bone has a lamellar structure because of the appositional bone development.

In mature foetuses osteons develop around the blood-vessels in the ventral and lateral part of the femur parallel to the longitudinal axis of the bone, and communicate with each other through side-canals. Osteons have their own walls. The matrix of the bone has a rough fibrous structure. The rough fibrous structure and zonelike lamellar structure (immature osteon system) are characteristic for young fetal bones. As a result of the resorptional and appositional ossification, the osteons, Haversian canals and the lamellar osseous system develop. The blood-vessels become spacious cavities on the place of the later Haversian canals. They appear first in the inner part of the bone in the neighborhood of the medullary cavity. The Haversian canals can be observed first on the back part of the femur, where the blood-vessels are wider from the beginning. They spread near the medullar cavity both in the inner and outer direction of the bone forward. Also the osseous structure is first formed on the back wall of the femur in its total thickness and the process is continued from here forward. At the age of 3, a ring consisting of a fairly wide, closed osseous structure can be observed around the medullary cavity.

Later the Haversian canals can be observed more and more often near the outer surface of the bone. However, there are no osteons around the blood-vessels in the homogeneous matrix below the periosteum in that period. In six-year-old children osteon tissue forms the majority of the bony wall of the femur. Balthazard and Müller (1921) also found that there is a considerable difference between the bone structures of the developing and developed individuals. The microscopic structure development of the fetal bones were studied on samples in chronological order. Fetal bones till the IVth lunar month were decalcified. Sections were made from the extremital bones older than VI lunar months.

Balthazard and Müller (1921) found mainly enchondral cartilaginous tissue on the transverse section at the histological study of the extremital bone of the foetus of II and a half lunar months. The periosteal bone layer is still narrow, the primary medullary spaces have already been formed and they are filled with cells. Resorption starts in the extremital bone of the foetus of III and half lunar months centrally and the first medullary canals appear. The enchondral bone laminates and the layers are situated concentrically. Medullary spaces regress and the number of anastomoses diminishes as well. On the place of the primary Haversian canals fissures develop. Ossification forms a coherent layer around the enchondral bone. The diaphysis is already enchondral in the cartilages (epiphysis) around the edges and it can be seen as periosteal bone-tissue, but no medullary space is formed at the ends of the diaphysis. Ossification can be observed ca. a month later in the distal parts of the epiphysis than in the diaphysis. The enchondral bone is restricted to the parts around the medullary space in the femur of the foetus of IV lunar months and it is clearly separated from the bone-tissue developed periosteally. The primary Haversian canals can be recognized as stretched fissures on the transverse sections of the extremital bones of the foetus of IV and a half lunar months. The enchondral bone tissue gradually decreases due to central resorption and periosteal bone development.

The rounded Haversian canals start to appear at the fetal age of V lunar months, and have larger dimensions than usually. The osteoclasts encircle the Haversian canal with thin concentric layers. The osteoblasts are situated in a more compact bony matrix in the bones of the foetus of VI lunar months. Concentric layers (laminae speciales) also appear here and there around the Haversian canals. The primary Haversian-osteon system has developed in the middle of the diaphysis at the age of VI and a half lunar months. Some Haversian canals can be seen still in the form of a wide cavity, but the majority are either round or oval.

However, the histological picture of the bones of the foetuses of X lunar months are different from the grown-up human bones in many respects. The differences are as follows: the Haversian canals can be observed in the extremital bones of the mature foetuses mainly on the edges below the periosteum. There are wide cavities in the middle of the wall-thickness (intermediary zone) as a consequence of the active resorption phenomena, which communicate with the medullary space. In the inner part the Haversian canals and the osseous lamellar system cannot be observed at all.

The introduction of the scanning electron microscopic studies led to new results in osteological research as well: Anderson 1967, Baud and Morgenthaler 1952, Cameron et al. 1964, Cameron 1967, Carlström 1957, Cooper et al. 1966, Cosslett 1962, Donath and Delling 1971, Engström 1960, Ortner 1967, Robinson et al. 1955, Wallgren 1957, West and Reed 1970, Wu et al. 1970. If the necessary instruments are available, this method has many advantages, as no circumstantial preparation of the bone samples, bone sections or decalcified segments is needed.

The SEM study can be carried out on the fracture surface of a very little piece of bone within an hour without any previous fixing.

The bone sample must be coated with a thin golden layer because of the difference of electron density. The authors would like to report about their observations in connection with the scanning electron microscopic studies in the present study, which was carried out to determine the fetal age.

MATERIALS AND METHODS

Bone samples of 25 human foetuses of different age (V–X lunar months old) from the bone collection of our institute, sawn-out in the middle part of the femur, were studied by scanning electron microscope. The bone samples were defatted and dehydrated in a mixture of ether and acetone and dried in an incubator at 37°C. The structure of the femur was studied on the transverse fracture surfaces. The bone samples were fixed on the preparate holding plates by the Colloida Silver (Polaron LTD, Watford, England) adhesive. The bone surface to be examined was coated by a 300 Å golden layer in the vacuum evaporator type Polaron SEM Coating Unit E 5100 at a vacuum of 0.02 tor and 20 mA intensity of current. SEM tests were carried out by the electron microscope type TESLA BS 300 at 25 kV accelerating voltage.

RESULTS

The changes due to age can be characterized in the extremital bone samples of the foetuses of different age by SEM study as follows. The age group characteristics clarified by traditional histological study can be traced well in the fetal bones by scanning electron microscopic studies, too.

Contrary to the bones of grown-up individuals, qualitative changes are prominent in determining the age instead of the evaluation of the quantitative changes. So the method of Kerley (1965), which can be applied for determining the age of the bones of grown-up individuals very well, cannot be used for fetal bones. By the way, no age determination method based on the evaluation of quantitative relations, similar to the above mentioned method, is known for fetal bones. The only basis and possibility to determine the age is to consider the essential moments of the process of ossification. The age group characteristics which have already been described by some authors, can be determined by scanning electron microscope, too.

The development of the structure of the fetal bones and their changes due to age are illustrated by SEM pictures. As the previous histological studies have already determined the age group characteristics of the development of the bone structure accurately, the most characteristic structural forms and changes are shown in chronological order instead of the SEM pictures. (*Figs 1-9*).

Figure 1. Transverse fracture surface of the femur of a foetus of five lunar months (No. 59, SEM 300×). The total bone width and wall thickness can be seen in the picture from the medullar edge to the outer surface. The lamellar bone tissue is enlarged. The majority of fissure-like blood-vessels are divided into circular zones. The vessel network (enlarged sinusoids) consists of vertical transverse, circular and radial parts. In the picture the bone structure is mainly broken by transverse and circular blood-vessels. At this fetal age there are no osteons yet.

The lamellar system can be observed in the form of circular layers considering the cross-section of the bone.

Figure 2. The enlarged picture of a transverse running vascular canal (No. 65, SEM 1000×). On the inner surface of the vessel-wall there are small openings (*canaliculi ossei*) and in the zone between the vascular

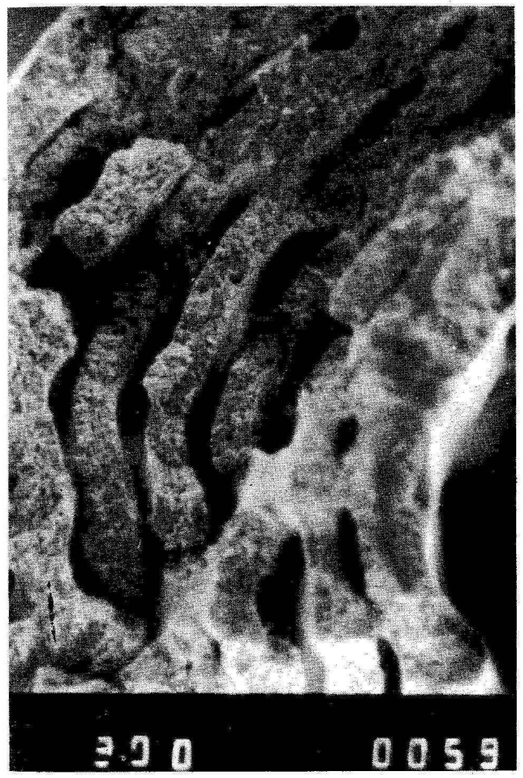
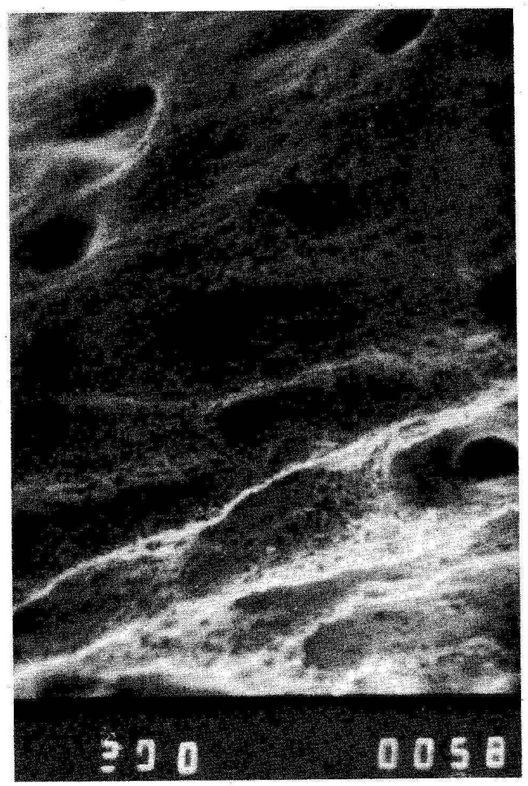


Figure 1.

Figure 2.



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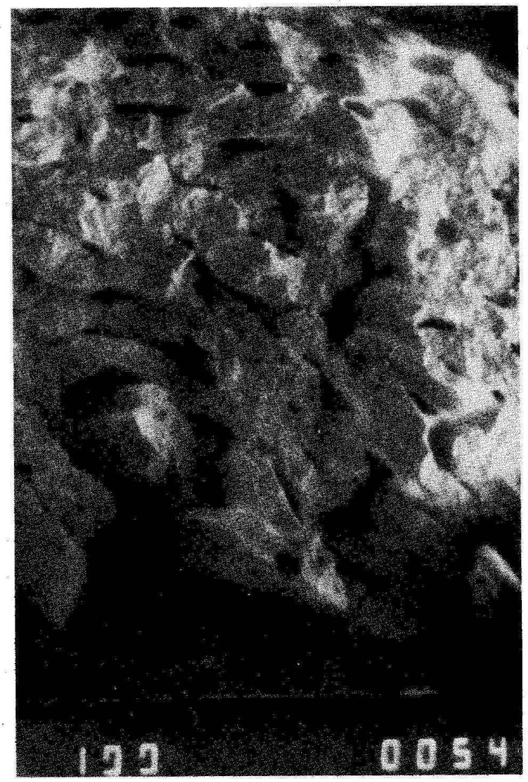

Figure 4.

canal cavities they are at the place of the osteocytes *(lacunae ossium)*.

Figure 3. The outer surface of the femur of a foetus of seven lunar months (No. 58, SEM 300×). The initial part of the blood-vessels below the periosteum, the openings of the so-called Volkmann canals can be seen.

Figure 4. Cross-sectional picture of the extremital bone of a foetus of seven lunar months – fracture surface (No. 54, SEM 100×). The Haversian canals are still fissure-like, not very regularly rounded, but they form a homogeneous structure in the outer 2/3 of the bone. Wider vascular canals can be observed only in the inner 1/3 of the cross-section, surrounded by bone trabeculae of changing dimensions.

Sometimes structures similar to osteon can be observed. They consist of circular Haversian canals mostly, around which either circular or polygonal bone trabeculae structures can be observed. This primitive osteon system differs from that of the grown-up individuals as there is no circular lamellar structure in the fetal osteons.

Figures 5 and 6 (No. 49 and 50; both of them SEM 1000×).

In the first figure an osteon structure chosen from the outer part of the extremital bone of a foetus of ten lunar months can be seen. The matrix between the two Haversian canals has a granular structure. It also shows the lacunae sporadically observed in the osteons.

It is remarkable that there is no circular lamellar structure around the Haversian canals.

Figure 6. A demonstration for the lack of the circular lamellar structure.

Figure 7. The cavities involving the osteocytes (lacunae ossium) can be seen in the femur of a male foetus of ten lunar months. Highly magnified (No. 51, SEM 3000×).

In the following two figures the great differences in the osteons of the fetal bones are shown.

The osteon of a male foetus of seven lunar months can be seen in *Figure 8*, which is considered „regular" (No. 57, SEM 1000×).

The osteon in the area near the medullar space of a female foetus of ten lunar months can be observed in *Figure 9*. Its enlarged Haversian canal of a sinusoid character and the surrounding trabecular bony substance are significantly different from those of grown-up individuals and the osteons observable at foetuses (No. 47, SEM 1000×).

DISCUSSION

The most important aspects which must be regarded at determining the age by the scanning electron microscopic study of bones are as follows:

1) The irregular structure of the inner bone part near the medullar space, the cross-section of the large

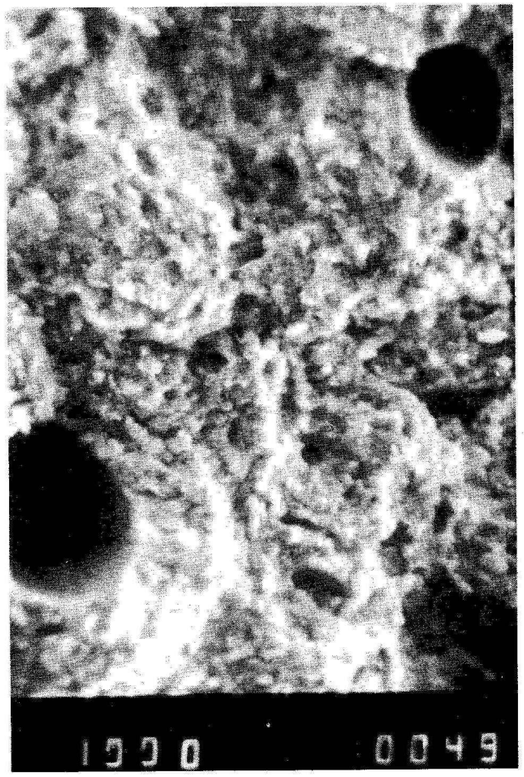

Figure 5.

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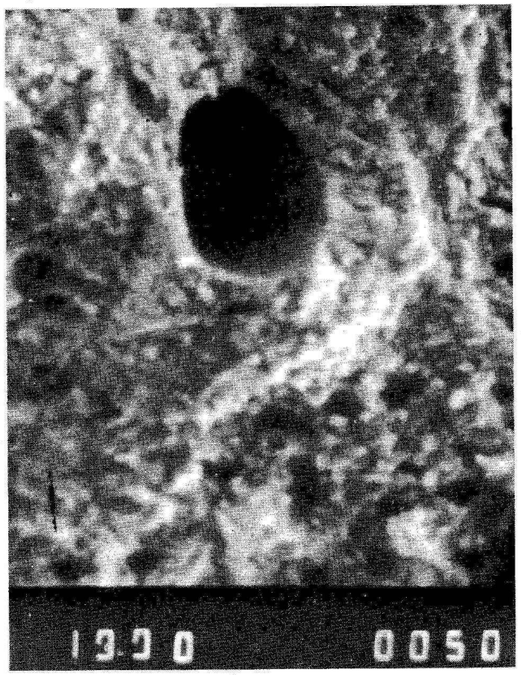

Figure 6.

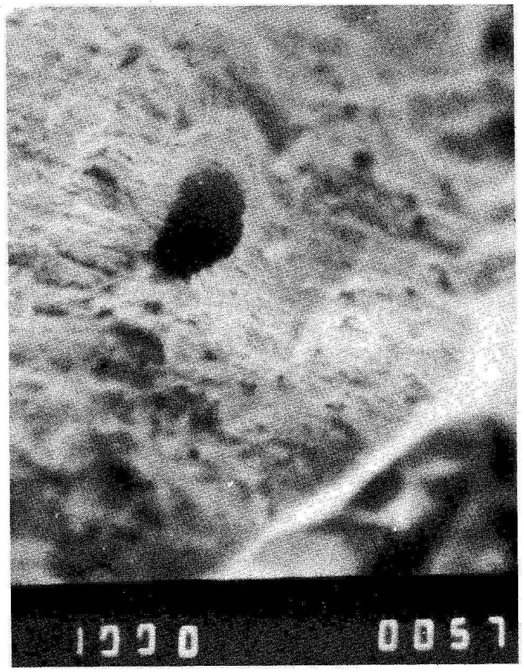

Figure 8.

Figure 9.

wide blood-vessels and irregularly shaped bone trabeculae around them refer to the early stage of maturity in the given foetus (younger than seven lunar months). All these morphological characteristics prove the immaturity of the foetus.

2) A circularly homogeneous structure can be observed in the area between the inner and outer surface of the femur in the SEM picture of the cross-section of the extremital bone of the foetuses of seven lunar months. The age group characteristics of this period is that the dimensions of the diameter of the Haversian canals and the bone trabeculae (primitive osteons) around them change significantly.

On the basis of the earlier light microscopic histological observations and the present SEM studies the conclusion can be drawn that it is impossible to determine the age of an unknown foetus with a lunar month age difference based on the study of the bone structure alone. But forensic medical statements can be made with approximate accuracy, serving the legal qualification of the crime in question (whether the foetus is mature or immature; whether abortion or early birth happened).

The significance of the SEM studies is not lesser from the aspect of determining „accurately" the age of the individual than the light microscopic histological, polarized optical and microradiographic study of the bone preparations (Clement et al. 1987).

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